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Differential repetitive DNA composition in the centromeric region of chromosomes of Amazonian lizard species in the family Teiidae

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Abstract

Differences in heterochromatin distribution patterns and its composition were observed in Amazonian teiid species. Studies have shown repetitive DNA harbors heterochromatic blocks which are located in centromeric and telomeric regions in *Ameiva ameiva* (Linnaeus, 1758), *Kentropyx calcarata* (Spix, 1825), *Kentropyx pelviceps* (Cope, 1868), and *Tupinambis teguixin* (Linnaeus, 1758). In *Cnemidophorus* sp.1, repetitive DNA has multiple signals along all chromosomes. The aim of this study was to characterize moderately and highly repetitive DNA sequences by C_0t1-DNA from *Ameiva ameiva* and *Cnemidophorus* sp.1 genomes through cloning and DNA sequencing, as well as mapping them chromosomally to better understand its organization and genome dynamics. The results of sequencing of DNA libraries obtained by C_0t1-DNA showed that different microsatellites, transposons, retrotransposons, and some gene families also comprise the fraction of repetitive DNA in the teiid species. FISH using C_0t1-DNA probes isolated from both *Ameiva ameiva* and *Cnemidophorus* sp.1 showed these sequences mainly located in heterochromatic centromeric, and telomeric regions in *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin* chromosomes, indicating they play structural and functional roles in the genome of these species. In *Cnemidophorus* sp.1, C_0t1-DNA probe isolated from *Ameiva ameiva* had multiple interstitial signals on chromosomes, whereas mapping of C_0t1-DNA isolated from the *Ameiva ameiva*

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and *Cnemidophorus* sp.1 highlighted centromeric regions of some chromosomes. Thus, the data obtained showed that many repetitive DNA classes are part of the genome of *Ameiva ameiva*, *Cnemidophorus* sp.1, *Kentroyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin*, and these sequences are shared among the analyzed teiid species, but they were not always allocated at the same chromosome position.

Keywords
centromere, C_0t1-DNA, FISH, heterochromatin, telomere

Introduction

Teiidae are a Neotropical lizard family characterized by karyotype diversity with a diploid number ranging from 34 to 52 chromosomes, as well as differences in heterochromatin composition and distribution patterns (Carvalho et al. 2015a, b). Amazonian teiid species exhibit considerable heterochromatic blocks located in centromeric and terminal regions of most chromosomes in *Ameiva ameiva* (Linnaeus, 1758), *Cnemidophorus* sp.1, *Kentropyx calcarata* (Spix, 1825) and *Kentropyx pelviceps* (Cope, 1868), whereas *Tupinambis teguixin* (Linnaeus, 1758) has few heterochromatic blocks in the centromeric regions of macrochromosomes, indicating differential heterochromatin distribution among teiid species (Carvalho et al. 2015a, b).

These heterochromatin blocks usually contain repetitive DNA, such as ribosomal DNA 5S, telomeric sequences, tropomyosin 1 genes, and the retrotransposons *Rex* 1 and *SINE* (Carvalho et al. 2015b). These repetitive elements have been mapped in chromosomes of *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin*. They were mainly located in heterochromatic centromeric and telomeric regions, and appeared to act on the structural organization of the centromere and/or telomere (Carvalho et al. 2015b). However, the composition of the heterochromatic fraction in the genome of *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin* was not restricted to ribosomal DNA 5S sequences, telomeric sequences, tropomyosin 1 gene, and retrotransposons *Rex* 1 and *SINE* because some chromosomes have heterochromatic blocks that are not hybridization signals of these repetitive elements (Carvalho et al. 2015a, b). In *Cnemidophorus* sp.1 the pattern of organization of these sequences is different from other teiids, presenting multiple signals along all chromosomes, with compartmentalized blocks mainly in interstitial chromosome regions (Carvalho et al. 2015b). This indicates differential composition of the centromeric region of this species.

Repetitive DNA may be isolated by various strategies, among them C_0t1-DNA is used to isolate total fraction of moderately and highly repetitive DNA sequences in the genome (Vicari et al. 2010). C_0t1-DNA is based on DNA re-association kinetics, where genome repetitive fractions tend to rapidly reanneal after total genomic DNA denaturation. Thus, average renaturation time of a particular sequence depends on the number of copies found in the genome and in time almost all DNA of a denatured sample will reassociate (Zwick et al. 1997, Ferreira and Martins 2008). Repetitive

DNA libraries of various species have identified sequences of microsatellites, satellites, ribosomal DNA, and transposable elements (transposons and retrotransposons) in the genome repetitive fraction (Hřibová et al. 2007, Zhang et al. 2012, Terencio et al. 2015). This DNA library enriched with moderate and highly repetitive sequences ($C_o t1$-DNA) may be mapped in chromosomes and sequenced, which helps in analysis and has aided in understanding the dynamic and genomic organization, in terms of the repetitive fraction, as well as in evolutionary processes (Ferreira and Martins 2008, Szinay et al. 2010, Yu et al. 2013).

The aim of this study was to characterize sequences of moderately and highly repetitive DNA sequences in *Ameiva ameiva* and *Cnemidophorus* sp.1 genomes. These teiid species have a large amount of heterochromatin that is organized differentially. Libraries enriched with repetitive DNA were cloned, sequenced, used as probes, and chromosomally mapped in *Ameiva ameiva*, *Cnemidophorus* sp.1, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin*. Furthermore, they assisted in the understanding of genomic sequence organization and dynamics in karyotypes of these Amazonian teiid species.

Material and methods

Samples of *Ameiva ameiva*, *Cnemidophorus* sp.1, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin* were collected in Amazonas State, Brazil, in different locations (Table 1). All of the collections were conducted with permission from the Brazilian Environmental Protection Agency (ICMBio/SISBIO 41825-1) (Table 1).

The animals were euthanized after capture in the field with a lethal dose of the anesthetic sodium thiopental to avoid being deprived of food or water. This research was approved by the Ethics Committee for Animal Experimentation of the Fundação Universidade do Amazonas/Universidade Federal do Amazonas (UFAM) (number 041/2013). No endangered or protected species were used in this research. The animals underwent cytogenetic procedures, were fixed with 10% formaldehyde (injected in the coelom and digestive tract), and preserved in 70% alcohol. Voucher specimens were deposited in the Herpetological Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA H31712, 33213, 34791, 34841, 35018). All samples were identified by the researcher Dr. Federico Arias.

Mitotic chromosomes were obtained from bone marrow cell suspensions *in vitro* using colchicine (Ford and Hamerton 1956). Because the larger heterochromatic regions in *Ameiva ameiva* and *Cnemidophorus* sp.1 represent divergence in the physical chromosomal mapping of different classes of repetitive DNA compared to other teiids, these two species were used to obtain a genomic library enriched with moderately and highly repetitive DNA, following the renaturation kinetics technique $C_o t1$-DNA (Zwick et al. 1997, Ferreira and Martins 2008). Genomic DNA samples from *Ameiva ameiva* and *Cnemidophorus* sp.1 (50 μl of 100–500 ng/μl of DNA in 0.3 M NaCl) were autoclaved (120°C) for 5 min to obtain fragments between 100 to 2000 bases pairs.

Table 1. Species of the Teinae and Tupinambinae subfamilies: collection sites, number and the analyzed animals and voucher specimens (lots) are listed. AM: Amazonas.

Subfamily	Species	Collection sites	Number and sex the analyzed animals	Voucher specimens (lots)
Teiinae	*Ameiva ameiva*	São Sebastião do Uatumã, AM Santa Isabel do Rio Negro, AM Tapauá, AM São Sebastião de Cuieiras, AM Reserva Adolpho Ducke, AM	30 (thirteen males; thirteen females; four without sex identification)	INPA H33213
	Cnemidophorus sp.1	Manaus, AM	13 (five males; eight females)	INPA H35018
	Kentropyx calcarata	São Sebastião do Uatumã, AM São Sebastião de Cuieiras, AM	7 (three males; four females)	INPA H31712
	Kentropyx pelviceps	Tapauá, AM	3 (three females)	INPA H34841
Tupinambinae	*Tupinambis teguixin*	São Sebastião do Uatumã, AM Tapauá, AM Reserva Adolpho Ducke, AM	5 (four females; one without sex identification)	INPA H34791

Then, samples were denatured at 95°C for 10 min, placed on ice for 10 seconds, and subsequently heated to reannealment at 65°C for 5 minutes. Thereafter, samples were incubated at 37°C for 8 minutes with a unit of S1 nuclease enzyme, whose function is to digest single-stranded DNA. Repetitive fraction of these samples was recovered by freezing in liquid nitrogen and subsequent DNA extraction using phenol-chloroform. The resulting DNA fragments were cloned and sequenced. C_0t1-DNA fragments of *Ameiva ameiva* and *Cnemidophorus* sp.1 were ligated into the plasmid vector pMOS-Blue blunt ended (GE Healthcare). Clones were sequenced in an automated ABI 3130 DNA sequencer XL (Applied Biosystem). The alignment of sequences was performed using the Clustal W tool (Thompson et al. 1994) included in the 7.0 BioEdit program (Hall 1999). The generated clones were submitted to BLASTN to detect similarity with public domain sequences contained in the NCBI database (http://www.ncbi.nlm.nih.gov), as well as in the Repbase database (Jurka et al. 2005) from the *Genetic Information Research Institute* (Giri) (http://www.girinst.org/repbase/), using the software CENSOR (Kohany et al. 2006).

C_0t1-DNA libraries were tagged using digoxigenin-11-dUTP for nick translation reaction, according to manufacturer's instructions (Dig-Nick Translation mix Roche). Anti-digoxigenin rhodamine (Roche) was used for signal detection. C_0t1-DNA libraries from *Ameiva ameiva* tagged using digoxigenin-11-dUTP were hybridized with chromosomes of the species. Further, homologous hybridizations were performed with Cot1-DNA libraries in *Cnemidophorus* sp.1. Probes obtained from C_0t1-DNA of *Ameiva ameiva* and C_0t1-DNA of *Cnemidophorus* sp.1 were also hybridized with chromosomes of other analyzed teiid species. FISH was performed under 77% stringency (2.5 ng/probe, 50% formamide, 10% dextran sulfate, and 2× SSC at 37°C for 18 h) (Pinkel et al. 1986). Chromosomes were counter stained with DAPI (2 mg/ml) in VectaShield mounting medium (Vector).

Chromosomes were analyzed using an epifluorescence microscope (Leica DFC 3000G). Metaphase stages were photographed; the karyotypes were loaded in Adobe Photoshop CS4 software and measured using Image J software. Afterward, the karyotypes were organized following the karyotype formula in karyotypes from *Ameiva ameiva*, *Kentropyx calcarata and Kentropyx pelviceps* were classified as gradual series of acrocentric chromosomes; those of *Cnemidophorus* sp.1 as biarmed, uniarmed, and microchromosomes; and those of *Tupinambis teguixin* as macro and microchromosomes.

Results

A total of 40 *Ameiva ameiva* Cot1-DNA clones were sequenced wherein 12 sequences corresponded 8 to microsatellites, 1 to transposons, 1 to retrotransposons, and 1 genes, all having high similarity with repetitive DNA deposited in public DNA banks (Table 2). For *Cnemidophorus* sp.1, 30 C_0t1-DNA clones sequenced wherein 8 sequences corresponded 6 to microsatellites, 1 to transposons and 1 genes, all also being highly similar to the repetitive DNAs deposited in public DNA banks (Table 3).

Table 2. Repetitive sequences obtained fraction C_0t1-DNA *Ameiva ameiva* with deposited sequences in the NCBI databases and GIRI.

Clone	Homology	Similarity	Identity
AA 1	DNA Transposons	Tc1-like de *Labeo rohita* (GenBank AY083617.1)	100%
AA 2	Microsatellite	*Betula platyphylla* var. *japonica* (GenBank AB084484.1)	100%
AA 3	Gene	TAP2 mRNA de *Oryzias latipes* (GenBank AB033382.1)	100%
AA 4	Microsatellite	*Coffea canephora* (GenBank EU526584.1)	100%
AA 5	Microsatellite	*Salmo salar* (GenBank Y11457.1)	96%
AA 6	Microsatellite	*Serranus cabrilla* (GenBank AM049431.1)	95%
AA 7	Microsatellite	*Hypericum perforatum* (GenBank FR732510.1)	93%
AA 8	Microsatellite	*Apteronemobius asahinai* (GenBank AB621739.1)	100%
AA 9	Non-LTR Retrotransposons	CR 1 (RepBase/GIRI*)	88%
AA 10	Microsatellite	*Bos taurus* (GenBank AF271953.1)	81%
AA 11	Microsatellite	*Colias behrii* (GenBank FN552755.1)	100%
AA 12	DNA Transposons	Tc1/mariner (RepBase/GIRI*)	80%

Table 3. Repetitive sequences obtained fraction C_0t1-DNA *Cnemidophorus* sp.1 with deposited sequences in the NCBI databases and GIRI.

Clone	Homology	Similarity	Identity
Cn 1	Gene	TAP2 mRNA de *Oryzias latipes* (GenBank AB033382.1)	100%
Cn 2	Microsatellite	*Betula platyphylla* var. *japonica* (GenBank AB084484.1)	100%
Cn 3	DNA Transposons	Tc1-like de *Labeo rohita* (GenBank AY083617.1)	100%
Cn 4	Microsatellite	*Colias behrii* (GenBank FN552755.1)	93%
Cn 5	Microsatellite	*Glaucosoma hebraicum* (GeneNank FJ409080.1)	97%
Cn 6	Microsatellite	*Colias behrii* (GenBank FN552755.1)	99%
Cn 7	Microsatellite	*Apteronemobius asahinai* (GenBank AB621739.1)	90%
Cn 8	Microsatellite	*Salmo salar* (GenBank Y11457.1)	96%

By using the homologous probe of C_0t1-DNA, *Ameiva ameiva* hybridization signals were located in the centromeric region/short arm of pairs 1 to 18, except pairs 9, 16, and 17, which showed interstitial signals (Figures 1a). When homologous hybridization of C_0t1-DNA *Cnemidophorus* sp.1 was performed with chromosomes of the species itself, signals were observed in the centromeric region/short arm of certain chromosomes, whereas the majority of chromosomes showed no signals in this chromosome region.

Hybrization using the heterologous probe of C_0t1-DNA obtained from *Ameiva ameiva* in *Cnemidophorus* sp.1 presented multiple signals along all chromosomes, with compartmentalized blocks mainly in interstitial regions (Figure 1b). In *Kentropyx calcarata* and *Kentropyx pelviceps*, signals were located at the centromeric region in the majority of chromosomes, with some pairs having terminal and interstitial signals (Figures 1c and 1d, respectively) and *Tupinambis teguixin* presenting signals in the

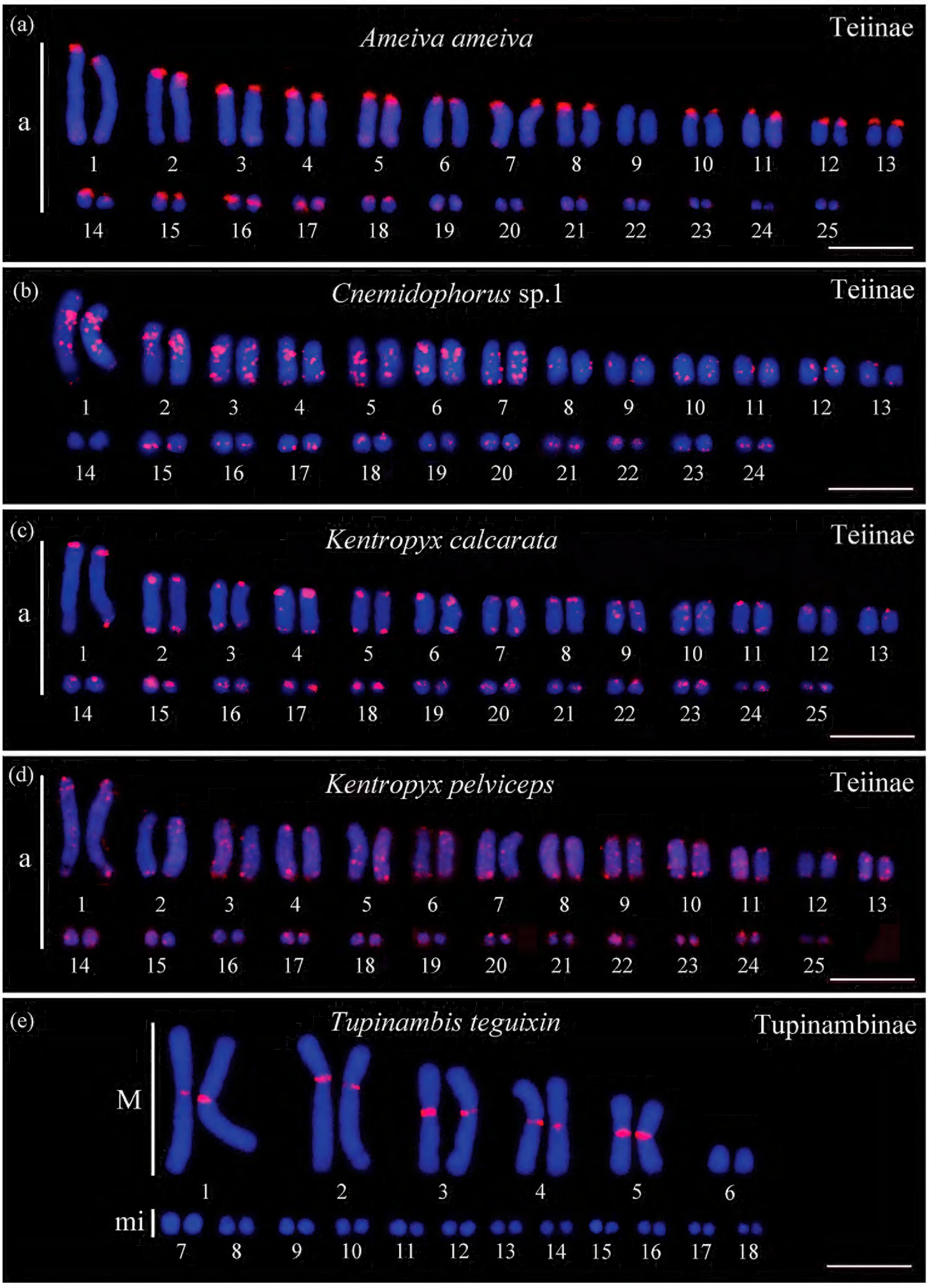

Figure 1. Karyotypes with *Ameiva ameiva* $C_o t$1-DNA probe hybridized (signal red). **a** *A. ameiva* **b** *Cnemidophorus* sp.1 **c** *Kentropyx calcarata* **d** *Kentropyx pelviceps* and **e** *Tupinambis teguixin*. The chromosomes were counterstained with DAPI. **a** = gradual series of acrocentric chromosomes. **m** = Macrochromosome, **mi** = microchromosome. Scale bar: 10 μm.

centromeric region in pairs 1, 2, 3, 4, and 5 (Figures 1e). Similar signal patterns were observed in heterologous hybridization of C_0t1-DNA obtained from *Cnemidophorus* sp.1 in chromosomes of *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin*; however, the signals were more tenuous.

Discussion

Several classes of repetitive DNA are included in the genome of Amazonian teiid species, such as ribosomal DNA 5S, telomeric sequences, tropomyosin 1 genes, and retrotransposons *Rex* 1 and *SINE*. Most of these repetitive DNA sequences are allocated to heterochromatin regions, in addition to acting structurally in the centromeric and telomeric organization for *Ameiva ameiva*, *Cnemidophorus* sp.1, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin* (Carvalho et al. 2015b). In addition to these functions, heterochromatin can have other activities in the genome. It can act in chromosome segregation, nuclear organization, mitosis regulation in cell cycle progression, cell proliferation, gene expression regulation, and may affect the process of gene recombination (Grewal and Jia 2007, Skipper 2007, Buhler 2009, Bloom 2014).

However, heterochromatin of teiids is not limited to ribosomal DNA 5S, telomeric sequences, tropomyosin 1 gene, retrotransposons *Rex* 1 and *SINE*, and presents a complex composition with various repetitive DNA. In libraries obtained by C_0t1-DNA sequencing, it was evidenced that different microsatellites, transposons, retrotransposons, some gene families and other type of sequences (e.g. satellite DNAs) are also present in this fraction of moderately and highly repetitive DNA. They were allocated preferentially to the centromeric and telomeric regions of *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin*. These sequences are also present in *Cnemidophorus* sp.1; however, they were present in euchromatic regions, similar to the pattern observed for telomeric sequences, tropomyosin 1 genes, and retrotransposons *Rex* 1 and *SINE*.

The sequences of repetitive DNA that were more abundant in moderately and highly repetitive fractions of the genome obtained using C_0t1-DNA in *Ameiva ameiva* and *Cnemidophorus* sp.1 were microsatellites, which were homologous with sequences of other organisms deposited in public databases, including plants, fish, mammals, bird and insect (Tables 2 and 3). However, the differences in microsatellites isolated from different species are noteworthy. Microsatellite accumulation may be associated with differentiation of sex chromosomes in some species of Sauropsida, because of high suppression of recombination, degeneration, and heterochromatinization (Pokorná et al. 2011, Gamble et al. 2014, Matsubara et al. 2015), but none of the species analyzed in this study had differentiated sex chromosomes; males and females had identical chromosomal constitution.

Microsatellite or simple sequence repeats (SSRs) are short sequences organized in long segments made up of tandem repeat units and are found in coding or non-coding regions in diverse species genomes (de Oliveira et al. 2015). In genetic analysis, they

are considered good molecular markers due to its high abundance in the genome, co-dominant inheritance, multi-allelic nature, good reproducibility, and has been used in studies of population genetics, phylogeny, linkage maps, and relationships (Qi et al. 2015, Čížková et al. 2015)

SSRs have functional roles in the genome, such as gene regulation, replication in transcription, protein function, and genome organization (Qi et al. 2015). These SSRs are generally located in centromeric regions and chromosomes ends of various organisms, such as those observed in *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin* in this study, which corroborates the results of other cytogenomic studies involving satellites/microsatellite DNA from some species of lizards (Lacertidae, Scincidae and Varanidae) and snakes (Colubridae, Pythonidae, and Viperidae). These studies showed that the SSRs are located in the heterochromatin region, specifically in the centromeric, pericentromeric, and/or telomeric regions in chromosomes, suggesting differences in the compositions of these regions in the Squamata genome (Singh et al. 1976, Grechko et al. 2005, Chaiprasertsri et al. 2013, Giovannotti et al. 2013, 2014, Matsubara et al. 2015).

In addition to microsatellites, about 50% of sequences obtained by C_0t1-DNA from two species, exhibited similarities to transposable elements (transposons and retrotransposons). One important characteristic of these transposable elements is the transposition mechanism; retrotransposons transpose via an intermediate from RNA and transposons move up the genome through DNA copies that may be contributing to diversity and plasticity of the genome during evolution (Kordis 2009, Kojima et al. 2015). Sequences presented similarity ranging from 80% to 100% with Tc1-like and Tc1/mariner DNA transposons. The Tc1 transposon is found in many eukaryotic genomes and moves into and/or across genomes. It is an element that has defective copies that have been carried across several mutations causing genetic element inactivation in the genome (Ivics and Izsvák 2015, Tellier et al. 2015). Tc1 are not highlighted in the active form in vertebrate genomes (Dornan et al. 2015, Ivics and Izsvák 2015, Tellier et al. 2015).

An retrotransposon, non-LTR retrotransposon CR1 (Chicken Repeat 1) was identified only in the *Ameiva ameiva* genome; however, this does not indicate that it was not present in the genome of *Cnemidophorus* sp.1, because it simply may not have been identified in this study. Retroelement CR1 is a LINE family that is widely distributed in various organisms, including vertebrates (birds, reptiles, and fish) and invertebrates (Thompson et al. 2009). CR1 contains a 5'UTR region, two reading frames (ORFs 1 and 2), and a terminal region 3'UTR. Terminal region 3'UTR have small repeats (microsatellites) relatively conserved within each of the seven CR1 groups (groups A to G) (Suh 2015). The CR1 groups are the only transposable elements that were active during the evolution of birds and reptiles, and thus, have been widely used as phylogenetic markers, in species identification, and for understanding genome evolution within Amniota (Suh et al. 2014, Suh 2015)

Repetitive DNA rDNA 5s, tropomyosin 1 genes, and retroelements *Rex* 1 and *SINE* (Carvalho et al. 2015b), have also been mapped on chromosomes of teiid species

analyzed in this study, nevertheless they were not highlighted in sequencing of moderately and highly repetitive DNA obtained by the C_0t1-DNA technique. C_0t1-DNA is product of DNA genomic concentration (C_0), renaturation time in seconds (t), and a constant that depends on buffer cation concentration (Britten and Kohne 1968, Britten et al. 1974). Still, the results of isolated sequences by this technique may be different due to differences in DNA fragmentation (which occur randomly) or cloning processes which may explain failure to obtain repetitive DNAs 5S rDNA tropomyosin 1 genes and retroelements Rex 1 and SINE that also comprise the repetitive fraction of the five analyzed teiid species.

Some clones presented similarity with part of the gene TAP-2 (transporter associated with antigen processing). TAP is encoded by class I major histocompatibility complex (MHC) genes and are responsible for the transport of antigen peptides from the cytoplasm to endoplasmic reticulum (Zhao et al. 2006, Murata et al. 2009). This carrier is comprised of TAP-1 and TAP-2 subunits and is essential in antigen processing and highly conserved among various eukaryotic species (Zhao et al. 2006, Murata et al. 2009). Some genes may be associated with repetitive DNA in different species genomes and may involve various functions, such as genome stability, gene expression regulation, chromatin formation, and as miRNAs (Roberts et al. 2014, Liang et al. 2015). This association of genes with repetitive DNA has been found in some species of lizards, fish, and mammals (Valente et al. 2011, Pokorná et al. 2011, Terencio et al. 2015), and is also evident for analyzed species in this study.

The physical chromosomal map of homologous probes and/or heterologous of C_0t1-DNA showing signals associated with heterochromatic regions in centromeric and telomeric regions in chromosomes of *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin*; and corroborated partially the standard heterochromatic (Carvalho et al. 2015a) the analyzed species. In addition, interstitial signals are also located in interstitial regions of the smaller pairs of chromosomes of *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin*, which confirm the presence of these sequences in this region. However, if we compare the location of these moderately and highly repetitive DNA obtained from C_0t1-DNA with other repetitive DNA already mapped to the same location (Carvalho et al. 2015b), the pattern of signals are similar, being allocated to heterochromatic regions in centromeric and telomeric regions of chromosomes of these teiid species. Although various classes of repetitive DNA can be located in the same chromosomal region from different species, the number of copies of each element may be different (Chaiprasertsri et al. 2013).

In *Cnemidophorus* sp.1, hybridization of moderately and highly repetitive sequences obtained from C_0t1-DNA from *Ameiva ameiva* presented multiple signals along chromosomes with compartmentalized blocks in interstitial regions, which probably are located in euchromatic regions since they are not located in centromeric and telomeric heterochromatic regions of chromosomes (Carvalho et al. 2015a, b). This pattern is similar to the distribution pattern of other repetitive DNA (Carvalho et al. 2015b), indicating that the pattern of chromosomal organization of repetitive elements is different

from the other analyzed teiid species. Moreover, repetitive DNA located in euchromatic regions may significantly influence regulatory regions a gene expression because distribution of repetitive elements in the genome, especially the high association to genes with metabolic function, might be the result of a positive selection during evolution and imply practical roles of these elements in gene functions (Wang et al. 2007, Liang et al. 2015). Studies with C_0t1-DNA demonstrated that although mouse and human genomes have similar families of repetitive elements, primary sequences differ significantly among genomes, the human C_0t1-DNA do not hybridize to chromosomes in mice and the same happens with human (Hall et al. 2014). Furthermore, C_0t1-DNA was hybridized on all euchromatic regions of chromosomes (Hall et al. 2014).

On the other hand, FISH using C_0t1-DNA homologous probe revealed signals mainly in centromeric regions of chromosomes of *Cnemidophorus* sp.1. Thus, heterochromatic centromeric fraction of *Cnemidophorus* sp.1 seems to be composed of microsatellites and transposable elements obtained by C_0t1-DNA of the specie itself and elucidated by its sequencing, being different of the sequences obtained by C_0t1-DNA of *Ameiva ameiva*, despite belonging to same categories. Yet C_0t1-DNA contains a variety of different sequences, including the satellite DNA which are among the major component of centromeric heterochromatin, conversely to TE or microsatellite which are interspersed along chromosomes. Other repetitive elements may be present in the heterochromatic fraction of this species, which have not been detected by C_0t1-DNA (Carvalho et al. 2015b). Further, in the centromeric region, other repetitive DNA centromere specifics (Rosic et al. 2014) may be present that may be allocated in both macrochromosomes and microchromosomes, since they share the same repetitive DNA (Giovannotti et al. 2014, Matsubara et al. 2015, this study).

Although its function and multiprotein components are conserved among organisms, these repetitive DNA are extremely divergent regarding its structure, organization, dynamics, and propagation mechanisms, influencing the diversification and evolution of centromeres (Plohl et al. 2014). Therefore, it is clear that several mechanisms may operate in the diversification of these regions, such as unequal crossing-over, gene conversion, changes mediated by transposable elements, and slippage of DNA polymerase replication, resulting in differential composition of heterochromatin in closely related species, among different chromosomes in the same species, or in species-specific chromosomes (Chaiprasertsri et al. 2013, Aldrup-MacDonald and Sullivan 2014, He et al. 2015, Gao et al. 2015).

Conclusion

This study contributes to understanding the heterochromatic fraction composition and structure and organization of repetitive DNA of teiid genomes and indicates that the different classes of moderately and highly repetitive DNA are part of *Ameiva ameiva*, *Cnemidophorus* sp.1, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin* genome. This means that these sequences are shared among the analyzed teiid species, although not

always allocated on the same chromosome region. Nevertheless, the physical mapping of repetitive DNA revealed similarity among the species *Ameiva ameiva*, *Kentropyx calcarata*, *Kentropyx pelviceps*, and *Tupinambis teguixin* and showed that the centromeric fraction of *Cnemidophorus* sp.1 is different from that of the other analyzed species.

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